



PCT10

# ENTERED

## RAW SEQUENCE LISTING

PATENT APPLICATION: US/10/069,040

DATE: 03/07/2002

TIME: 15:35:09

Input Set : A:\pto.vsk.txt

Output Set: N:\CRF3\03072002\J069040.raw

3 <110> APPLICANT: Novozymes A/S  
 4 Svendsen, Allan  
 5 Andersen, Carsten  
 6 Borchert, Torben Vedel  
 8 <120> TITLE OF INVENTION: Pullulanase variants and methods for preparing such variants  
 with 9 predetermined properties  
 11 <130> FILE REFERENCE: 6072.204-US  
 C--> 13 <140> CURRENT APPLICATION NUMBER: US/10/069,040  
 C--> 14 <141> CURRENT FILING DATE: 2002-02-19  
 16 <160> NUMBER OF SEQ ID NOS: 40  
 18 <170> SOFTWARE: PatentIn version 3.1  
 20 <210> SEQ ID NO: 1  
 21 <211> LENGTH: 2766  
 22 <212> TYPE: DNA  
 23 <213> ORGANISM: Bacillus acidopullulyticus  
 25 <220> FEATURE:  
 26 <221> NAME/KEY: CDS  
 27 <222> LOCATION: (1)..(2766)  
 28 <223> OTHER INFORMATION:  
 31 <400> SEQUENCE: 1  
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 33 Asp Ser Thr Ser Thr Lys Val Ile Val His Tyr His Arg Phe Asp Ser  
 34 1 5 10 15  
 36 aac tat acg aat tgg gac gtc tgg atg tgg cct tat cag cct gtt aat 96  
 37 Asn Tyr Thr Asn Trp Asp Val Trp Met Trp Pro Tyr Gln Pro Val Asn  
 38 20 25 30  
 40 ggt aat gga gca gct tac caa ttc act ggt aca aat gat gat ttt ggc 144  
 41 Gly Asn Gly Ala Ala Tyr Gln Phe Thr Gly Thr Asn Asp Asp Phe Gly  
 42 35 40 45  
 44 gct gtt gca gat acg caa gtg cct gga gat aat aca caa gtt ggt ttg 192  
 45 Ala Val Ala Asp Thr Gln Val Pro Gly Asp Asn Thr Gln Val Gly Leu  
 46 50 55 60  
 48 att gtt cgt aaa aat gat tgg agc gag aaa aat aca cca aac gat ctc 240  
 49 Ile Val Arg Lys Asn Asp Trp Ser Glu Lys Asn Thr Pro Asn Asp Leu  
 50 65 70 75 80  
 52 cat att gac ctt gca aaa ggc cat gaa gta tgg att gta caa ggg gat 288  
 53 His Ile Asp Leu Ala Lys Gly His Glu Val Trp Ile Val Gln Gly Asp  
 54 85 90 95  
 56 cca act att tat tac aat ctg agc gac gca cag gct gcc gca ata cca 336  
 57 Pro Thr Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ala Ile Pro  
 58 100 105 110  
 60 tct gtt tca aat gcc tat ctt gat gat gaa aaa aca gta cta gca aag 384  
 61 Ser Val Ser Asn Ala Tyr Leu Asp Asp Glu Lys Thr Val Leu Ala Lys

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62	115	120	125	
64	cta agt atg ccg atg acg ctg gcg gat gct gca agc ggc ttt acg gtt	432		
65	Leu Ser Met Pro Met Thr Leu Ala Asp Ala Ala Ser Gly Phe Thr Val			
66	130	135	140	
68	ata gat aaa acc aca ggt gaa aaa atc cct gtc acc tct gct gta tcc	480		
69	Ile Asp Lys Thr Thr Gly Glu Lys Ile Pro Val Thr Ser Ala Val Ser			
70	145	150	155	160
72	gca aat ccg gta act gcc gtt ctt gtt gga gat tta caa cag gct ttg	528		
73	Ala Asn Pro Val Thr Ala Val Leu Val Gly Asp Leu Gln Gln Ala Leu			
74	165	170	175	
76	gga gca gcg aat aat tgg tca cca gat gat gat cac aca ctg cta aaa	576		
77	Gly Ala Ala Asn Asn Trp Ser Pro Asp Asp Asp His Thr Leu Leu Lys			
78	180	185	190	
80	aag ata aat cca aac ctt tac caa tta tcg ggg aca ctt cca gct ggt	624		
81	Lys Ile Asn Pro Asn Leu Tyr Gln Leu Ser Gly Thr Leu Pro Ala Gly			
82	195	200	205	
84	aca tac caa tat aag ata gcc ttg gac cat tct tgg aat acc tcc tat	672		
85	Thr Tyr Gln Tyr Lys Ile Ala Leu Asp His Ser Trp Asn Thr Ser Tyr			
86	210	215	220	
88	cca ggt aac aat gta agt ctt act gtt cct cag gga ggg gaa aag gtt	720		
89	Pro Gly Asn Asn Val Ser Leu Thr Val Pro Gln Gly Gly Glu Lys Val			
90	225	230	235	240
92	acc ttt acc tat att cca tct acc aac cag gta ttc gat agc gtc aat	768		
93	Thr Phe Thr Tyr Ile Pro Ser Thr Asn Gln Val Phe Asp Ser Val Asn			
94	245	250	255	
96	cat cct aac caa gca ttc cct aca tcc tca gca ggg gtc cag aca aat	816		
97	His Pro Asn Gln Ala Phe Pro Thr Ser Ser Ala Gly Val Gln Thr Asn			
98	260	265	270	
100	tta gtc caa ttg act tta gcg agt gca ccg gat gtc acc cat aat tta	864		
101	Leu Val Gln Leu Thr Leu Ala Ser Ala Pro Asp Val Thr His Asn Leu			
102	275	280	285	
104	gat gta gca gca gac ggt tac aaa gcg cac aat att tta cca agg aat	912		
105	Asp Val Ala Ala Asp Gly Tyr Lys Ala His Asn Ile Leu Pro Arg Asn			
106	290	295	300	
108	gtt tta aat ctg ccg cgg tat gat tat agt gga aat gat ttg ggt aat	960		
109	Val Leu Asn Leu Pro Arg Tyr Asp Tyr Ser Gly Asn Asp Leu Gly Asn			
110	305	310	315	320
112	gtt tat tca aag gat gca aca tcc ttc cgg gta tgg gct cca aca gct	1008		
113	Val Tyr Ser Lys Asp Ala Thr Ser Phe Arg Val Trp Ala Pro Thr Ala			
114	325	330	335	
116	tcg aat gtc cag ttg ctt tta tac aat agt gag aaa ggt tca ata act	1056		
117	Ser Asn Val Gln Leu Leu Leu Tyr Asn Ser Glu Lys Gly Ser Ile Thr			
118	340	345	350	
120	aaa cag ctt gaa atg caa aag agt gat aac ggt aca tgg aaa ctt cag	1104		
121	Lys Gln Leu Glu Met Gln Lys Ser Asp Asn Gly Thr Trp Lys Leu Gln			
122	355	360	365	
124	gtt tct ggt aat ctt gaa aac tgg tat tat cta tat caa gtc aca gtg	1152		
125	Val Ser Gly Asn Leu Glu Asn Trp Tyr Tyr Leu Tyr Gln Val Thr Val			
126	370	375	380	

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128	aat	ggg	aca	aca	caa	acg	gca	gtt	gat	cca	tat	gcg	cgt	gct	att	tct	1200
129	Asn	Gly	Thr	Thr	Gln	Thr	Ala	Val	Asp	Pro	Tyr	Ala	Arg	Ala	Ile	Ser	
130	385					390					395					400	
132	gtc	aat	gca	aca	cgc	ggg	atg	att	gtg	gac	cta	aaa	gct	acc	gat	cct	1248
133	Val	Asn	Ala	Thr	Arg	Gly	Met	Ile	Val	Asp	Leu	Lys	Ala	Thr	Asp	Pro	
134					405					410					415		
136	gca	ggg	tgg	cag	gga	gat	cat	gaa	cag	aca	cct	gcg	aat	cca	gta	gat	1296
137	Ala	Gly	Trp	Gln	Gly	Asp	His	Glu	Gln	Thr	Pro	Ala	Asn	Pro	Val	Asp	
138				420					425					430			
140	gaa	gtg	att	tat	gaa	gcg	cat	gta	cgc	gat	ttt	tcg	att	gat	gct	aat	1344
141	Glu	Val	Ile	Tyr	Glu	Ala	His	Val	Arg	Asp	Phe	Ser	Ile	Asp	Ala	Asn	
142			435					440					445				
144	tca	ggg	atg	aaa	aat	aaa	ggg	aag	tat	tta	gcg	ttt	aca	gag	cat	gga	1392
145	Ser	Gly	Met	Lys	Asn	Lys	Gly	Lys	Tyr	Leu	Ala	Phe	Thr	Glu	His	Gly	
146		450					455					460					
148	aca	aaa	gga	ccg	gat	cat	gta	aag	aca	ggg	att	gat	agt	ttg	aag	gaa	1440
149	Thr	Lys	Gly	Pro	Asp	His	Val	Lys	Thr	Gly	Ile	Asp	Ser	Leu	Lys	Glu	
150	465					470					475					480	
152	ttg	ggc	atc	acc	act	gtt	caa	ttg	caa	cct	gtt	gag	gag	ttt	aac	agt	1488
153	Leu	Gly	Ile	Thr	Thr	Val	Gln	Leu	Gln	Pro	Val	Glu	Glu	Phe	Asn	Ser	
154					485					490					495		
156	att	gat	gag	acc	cag	cct	gat	acg	tat	aac	tgg	ggc	tac	gat	cca	agg	1536
157	Ile	Asp	Glu	Thr	Gln	Pro	Asp	Thr	Tyr	Asn	Trp	Gly	Tyr	Asp	Pro	Arg	
158				500					505					510			
160	aac	tat	aac	gta	cca	gag	gga	gct	tat	gcc	aca	act	cca	gaa	gga	aca	1584
161	Asn	Tyr	Asn	Val	Pro	Glu	Gly	Ala	Tyr	Ala	Thr	Thr	Pro	Glu	Gly	Thr	
162			515					520					525				
164	gcg	cgt	ata	aca	gaa	tta	aag	caa	tta	att	caa	agc	ctt	cat	cag	cag	1632
165	Ala	Arg	Ile	Thr	Glu	Leu	Lys	Gln	Leu	Ile	Gln	Ser	Leu	His	Gln	Gln	
166		530					535					540					
168	cgg	att	ggg	gtc	aat	atg	gat	gtt	gtt	tat	aac	cat	acc	ttt	gat	gtg	1680
169	Arg	Ile	Gly	Val	Asn	Met	Asp	Val	Val	Tyr	Asn	His	Thr	Phe	Asp	Val	
170	545					550					555					560	
172	atg	gtt	tct	gat	ttt	gat	aaa	att	gtc	ccg	caa	tat	tat	tat	cgt	acc	1728
173	Met	Val	Ser	Asp	Phe	Asp	Lys	Ile	Val	Pro	Gln	Tyr	Tyr	Tyr	Arg	Thr	
174					565					570					575		
176	gat	agt	aat	ggc	aat	tat	acg	aac	gga	tca	ggg	tgc	ggc	aat	gaa	ttc	1776
177	Asp	Ser	Asn	Gly	Asn	Tyr	Thr	Asn	Gly	Ser	Gly	Cys	Gly	Asn	Glu	Phe	
178				580					585					590			
180	gcg	act	gag	cat	cca	atg	gca	caa	aag	ttt	gtg	ctt	gat	tca	gtt	aat	1824
181	Ala	Thr	Glu	His	Pro	Met	Ala	Gln	Lys	Phe	Val	Leu	Asp	Ser	Val	Asn	
182			595					600					605				
184	tat	tgg	gta	aat	gag	tac	cac	gtg	gat	ggc	ttc	cgt	ttt	gac	tta	atg	1872
185	Tyr	Trp	Val	Asn	Glu	Tyr	His	Val	Asp	Gly	Phe	Arg	Phe	Asp	Leu	Met	
186		610					615				620						
188	gct	ctt	tta	gga	aaa	gac	acg	atg	gca	aaa	ata	tca	aac	gag	ctg	cat	1920
189	Ala	Leu	Leu	Gly	Lys	Asp	Thr	Met	Ala	Lys	Ile	Ser	Asn	Glu	Leu	His	
190	625					630					635					640	
192	gcc	att	aat	cct	ggg	att	gtt	tta	tat	gga	gaa	cca	tgg	act	ggc	ggc	1968

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194					645					650					655		
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197	Thr	Ser	Gly	Leu	Ser	Ser	Asp	Gln	Leu	Val	Thr	Lys	Gly	Gln	Gln	Lys	
198				660					665					670			
200	gga	tta	gga	att	ggc	gtt	ttc	aac	gat	aat	ata	cgt	aat	ggg	ctc	gat	2064
201	Gly	Leu	Gly	Ile	Gly	Val	Phe	Asn	Asp	Asn	Ile	Arg	Asn	Gly	Leu	Asp	
202			675					680					685				
204	ggg	aac	gtg	ttt	gat	aaa	acg	gca	caa	ggc	ttt	gca	aca	gga	gat	cca	2112
205	Gly	Asn	Val	Phe	Asp	Lys	Thr	Ala	Gln	Gly	Phe	Ala	Thr	Gly	Asp	Pro	
206		690					695					700					
208	aac	cag	gtg	gat	gtc	att	aaa	aat	gga	gta	atc	ggt	agt	att	caa	gat	2160
209	Asn	Gln	Val	Asp	Val	Ile	Lys	Asn	Gly	Val	Ile	Gly	Ser	Ile	Gln	Asp	
210	705					710					715				720		
212	ttt	act	tca	gca	cct	agc	gaa	acg	att	aac	tat	gtt	aca	agc	cat	gat	2208
213	Phe	Thr	Ser	Ala	Pro	Ser	Glu	Thr	Ile	Asn	Tyr	Val	Thr	Ser	His	Asp	
214				725					730					735			
216	aac	atg	acg	ctt	tgg	gat	aaa	att	tta	gca	agt	aat	cca	agt	gac	act	2256
217	Asn	Met	Thr	Leu	Trp	Asp	Lys	Ile	Leu	Ala	Ser	Asn	Pro	Ser	Asp	Thr	
218				740					745				750				
220	gag	gct	gac	cga	att	aaa	atg	gat	gaa	ttg	gca	cat	gcc	gta	gta	ttc	2304
221	Glu	Ala	Asp	Arg	Ile	Lys	Met	Asp	Glu	Leu	Ala	His	Ala	Val	Val	Phe	
222			755				760					765					
224	act	tca	caa	ggt	gta	cca	ttt	atg	caa	ggt	gga	gaa	gaa	atg	ctg	agg	2352
225	Thr	Ser	Gln	Gly	Val	Pro	Phe	Met	Gln	Gly	Gly	Glu	Glu	Met	Leu	Arg	
226		770					775					780					
228	aca	aaa	ggc	gga	aat	gat	aac	agt	tat	aac	gct	gga	gat	agt	gtg	aat	2400
229	Thr	Lys	Gly	Gly	Asn	Asp	Asn	Ser	Tyr	Asn	Ala	Gly	Asp	Ser	Val	Asn	
230	785				790					795					800		
232	cag	ttc	gac	tgg	tca	aga	aag	gcg	caa	ttt	aag	gat	gtt	ttt	gac	tac	2448
233	Gln	Phe	Asp	Trp	Ser	Arg	Lys	Ala	Gln	Phe	Lys	Asp	Val	Phe	Asp	Tyr	
234				805					810					815			
236	ttt	tct	agt	atg	att	cat	ctt	cgt	aat	cag	cac	ccg	gca	ttc	agg	atg	2496
237	Phe	Ser	Ser	Met	Ile	His	Leu	Arg	Asn	Gln	His	Pro	Ala	Phe	Arg	Met	
238				820					825				830				
240	acg	aca	gcg	gat	caa	att	aaa	cag	aat	ctt	aca	ttc	tta	gaa	agc	cca	2544
241	Thr	Thr	Ala	Asp	Gln	Ile	Lys	Gln	Asn	Leu	Thr	Phe	Leu	Glu	Ser	Pro	
242			835				840					845					
244	aca	aac	acg	gta	gct	ttc	gag	tta	aag	aat	tat	gca	aac	cat	gat	aca	2592
245	Thr	Asn	Thr	Val	Ala	Phe	Glu	Leu	Lys	Asn	Tyr	Ala	Asn	His	Asp	Thr	
246		850					855					860					
248	tgg	aaa	aat	ata	att	gtc	atg	tat	aac	cca	aat	aag	act	tcc	caa	acc	2640
249	Trp	Lys	Asn	Ile	Ile	Val	Met	Tyr	Asn	Pro	Asn	Lys	Thr	Ser	Gln	Thr	
250	865				870					875					880		
252	ctt	aat	cta	cca	agt	gga	gat	tgg	acc	att	gta	gga	ttg	gga	gat	caa	2688
253	Leu	Asn	Leu	Pro	Ser	Gly	Asp	Trp	Thr	Ile	Val	Gly	Leu	Gly	Asp	Gln	
254				885					890				895				
256	ata	ggt	gag	aaa	tca	tta	ggg	cat	gta	atg	ggt	aat	gtt	caa	gta	ccg	2736
257	Ile	Gly	Glu	Lys	Ser	Leu	Gly	His	Val	Met	Gly	Asn	Val	Gln	Val	Pro	

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266 <211> LENGTH: 921
267 <212> TYPE: PRT
268 <213> ORGANISM: Bacillus acidopullulyticus
270 <400> SEQUENCE: 2
272 Asp Ser Thr Ser Thr Lys Val Ile Val His Tyr His Arg Phe Asp Ser
273 1          5          10          15
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277          20          25          30
280 Gly Asn Gly Ala Ala Tyr Gln Phe Thr Gly Thr Asn Asp Asp Phe Gly
281          35          40          45
284 Ala Val Ala Asp Thr Gln Val Pro Gly Asp Asn Thr Gln Val Gly Leu
285          50          55          60
288 Ile Val Arg Lys Asn Asp Trp Ser Glu Lys Asn Thr Pro Asn Asp Leu
289 65          70          75          80
292 His Ile Asp Leu Ala Lys Gly His Glu Val Trp Ile Val Gln Gly Asp
293          85          90          95
296 Pro Thr Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ala Ile Pro
297          100         105         110
300 Ser Val Ser Asn Ala Tyr Leu Asp Asp Glu Lys Thr Val Leu Ala Lys
301          115         120         125
304 Leu Ser Met Pro Met Thr Leu Ala Asp Ala Ala Ser Gly Phe Thr Val
305          130         135         140
308 Ile Asp Lys Thr Thr Gly Glu Lys Ile Pro Val Thr Ser Ala Val Ser
309 145         150         155         160
312 Ala Asn Pro Val Thr Ala Val Leu Val Gly Asp Leu Gln Gln Ala Leu
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316 Gly Ala Ala Asn Asn Trp Ser Pro Asp Asp Asp His Thr Leu Leu Lys
317          180         185         190
320 Lys Ile Asn Pro Asn Leu Tyr Gln Leu Ser Gly Thr Leu Pro Ala Gly
321          195         200         205
324 Thr Tyr Gln Tyr Lys Ile Ala Leu Asp His Ser Trp Asn Thr Ser Tyr
325          210         215         220
328 Pro Gly Asn Asn Val Ser Leu Thr Val Pro Gln Gly Gly Glu Lys Val
329 225         230         235         240
332 Thr Phe Thr Tyr Ile Pro Ser Thr Asn Gln Val Phe Asp Ser Val Asn
333          245         250         255
336 His Pro Asn Gln Ala Phe Pro Thr Ser Ser Ala Gly Val Gln Thr Asn
337          260         265         270
340 Leu Val Gln Leu Thr Leu Ala Ser Ala Pro Asp Val Thr His Asn Leu
341          275         280         285
344 Asp Val Ala Ala Asp Gly Tyr Lys Ala His Asn Ile Leu Pro Arg Asn
345          290         295         300
348 Val Leu Asn Leu Pro Arg Tyr Asp Tyr Ser Gly Asn Asp Leu Gly Asn
349 305         310         315         320

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L:13 M:270 C: Current Application Number differs, Replaced Current Application Number

L:14 M:271 C: Current Filing Date differs, Replaced Current Filing Date